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STUDIES ON THE HILL REACTION ACTIVITY OF SOLUBLE CHLOROPLAST EXTRACTS

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## Introduction

This study is concerned with the mechanisms and essential reactants of that part of the photosynthetic process in which oxygen is produced by photolysis of water. This is essentially the light-absorbing reaction, which can be studied in vitro separately from the carbon dioxide-fixing reactions. The photolysis of water and evolution of oxygen that occurs when chloroplasts or fragments of them are illuminated in the presence of a suitable electron acceptor is generally referred to as the Hill reaction. Attempts to reduce the Hill reaction system to a nonparticulate state have generally resulted in loss of activity. Progress toward this goal would be useful in defining the minimum components and conditions required for photolytic activity, as well as in determining the details of the mechanisms of energy transfer. Recent experiments along these lines in our laboratories led to establishment of this project to study chloroplast extracts.

The long range plan of study includes analytical investigations to identify and characterize the components of the photoactive complex, and to determine the function of each; examination of the role of proteins, especially enzymes, in the complex; studies on methods of preparing extracts of chloroplasts and on the stability of the preparations; determination of the participation and role of various cofactors; and application of spectroscopic and other physical methods to elucidation of the characteristics of the system.

## Activity During the Period

A transition was made from the manometric method of measuring Hill reaction activity to the polarographic method. Chloroplast fragment preparations were studied for oxygen-evolving activity as a function of the amount of biocatalyst.

Some laboratory modifications that were made included moving the Elphor high-voltage, carrier-free electrophoresis instrument to a special darkened laboratory adjacent to where the Hill reaction activity and spectrophotometry are conducted. Air-conditioning was installed to facilitate the operation of the Elphor unit.

## Plans

Preparative electrophoresis studies will be continued and augmented by preliminary centrifugal fractionation of chloroplast fragment suspensions. Improvements in the polarographic method of oxygen sensing will be pursued.

## Experimental

During this quarter, we changed from the manometric to the polarographic electrode method of measuring Hill reaction activity. The polarographic electrode technique, pioneered by Blinks and co-workers, has the advantage

of continuous measurement of oxygen evolution during the Hill reaction run. Further improvements being considered should provide even greater sensitivity over manometric methods.

A Beckman oxygen sensor (39065) and adapter box (96260) were used in conjunction with a Model 76 Beckman expanded scale pH meter. After the equipment was set up, several minor adjustments had to be made to adapt it to use with a potentiometric recorder. The shorting strap across the recorder output on the pH meter was replaced with a resistor. Using a 1-mv recorder (Servo/Riter Model PSR, Texas Instruments, Inc.) with an attenuation path on the input circuit, a 49.5-ohm resistor,  $\pm 1$  percent,  $\frac{1}{4}$  watt, gave a 10-mv recorder sensitivity. The recorder span was adjusted to read 75  $\mu$ l of oxygen evolved per chart division. (Note: the technical assistance of Franklin M. Church during the installation of the new instrumentation is gratefully acknowledged.)

Experiments were conducted to compare the accuracy of the results obtained using the polarographic electrode with the Warburg respirometer. Spinach chloroplasts were prepared as described in Quarterly Report No. 5. The oxygen-sensing polarographic electrode was calibrated in air using the X10 attenuation and the 100 percent scale on the oxygen adapter box. This gave a reading of 21 percent partial pressure of oxygen in air on the pH meter; the recorder was adjusted to read accordingly.

The electrode was inserted in a modified Warburg flask. An additional side tube was attached to the flask to facilitate flushing with prepurified nitrogen to obtain an anaerobic base line. The flask contained 1.0 ml of chloroplast fragment suspension in the side arm, 1.0 ml of 0.01 M potassium ferricyanide solution and 0.8 ml of 0.025 M Tris buffer (pH 7.56) in the main portion of the flask, and 0.2 ml of 10 percent potassium hydroxide solution in the center well.

The flask was immersed in the constant temperature water bath ( $14.4 \pm 0.1^\circ\text{C}$ ) of the Warburg apparatus (Model 5-134, American Instrument, Inc.) and shaken while being flushed with prepurified nitrogen. After approximately 20 minutes, a steady baseline was obtained on the recorder. The solutions were then mixed and allowed to stabilize in the dark for a few more minutes before the light was turned on to drive the photochemical reaction. Comparable flasks, including a thermobarometer, were run using standard manometric methods. The results by both methods compared favorably. In one run, the microliters of oxygen evolved per minute were 4.43 (manometric) and 4.5 (polarographic). In another experiment, they were 5.94 and 6.3, respectively.

This method of sensing the evolution of oxygen was used in a series of experiments in which the Hill reaction activity was determined as a function of the volume of chloroplast fragment suspension added as the biocatalyst. The results for a series of preparations are summarized in Table I.

Table I

HILL REACTION ACTIVITIES OF CHLOROPLAST FRAGMENT PREPARATIONS MEASURED  
WITH THE POLAROGRAPHIC OXYGEN-SENSING ELECTRODE<sup>a</sup>

Experiment Number	$Q_{O_2}^{Chl}$			
	Volume of Chloroplast Suspension (ml) <sup>b</sup>			
	1.00	0.50	0.25	0.125
1	339	528	---	---
2	---	323	456	---
3	---	150	286	---
4	536	562	715	---
5	378	510	641	782
6	268	---	524	---

<sup>a</sup> Influenced by the quantity of biocatalyst added to the reaction mixture

<sup>b</sup> All experiments were conducted with a total volume of solution of 3.00 ml, in the 15-ml Warburg flasks.

### Discussion

In general, the slope of the curve of Hill reaction activity is a function of chlorophyll concentration in the system and indicates increasing activity as the concentration of biocatalyst approaches infinite dilution. Unfortunately, the data are too incomplete to permit useful evaluation. In future experiments we plan to study the variation of activity at a fixed level of chlorophyll concentration, e.g., 0.50 or 0.25 mg chlorophyll per milliliter, and to study the shape of the curve in greater detail at lower concentrations of chlorophyll.

One explanation frequently offered is that some chloroplast fragments screen others from receiving adequate illumination. Consequently, the Hill reaction activity is proportional to the efficiency of exposure of the biocatalyst. This would be improved at higher dilutions at a sacrifice of precision. However, there should be a concentration below which the activity is a linear function of the biocatalyst concentration, i.e., free of screening interference.

Another possibility is suggested by the Schutz rule--it is necessary to quadruple the quantity of enzyme in order to double the rate of reaction (J. B. Sumner and G. F. Somers, Chemistry and Methods of Enzymes, 3rd ed., 1953, Academic Press, p. 17). Assuming that the concerted enzymatic activity in the Hill reaction is directly related to the

quantity of chlorophyll, the results very closely resemble those typical of the Schutz phenomenon. Further studies of the reproducibility of results will help in the adoption of this interpretation.

The studies with the polarographic electrode were performed with a single flask and its electrode. It should be possible to monitor several flasks simultaneously through a multipoint recorder.



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